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MOLECULAR EVOLUTIONARY ANALYSIS OF PADDY PEST, *COFANA SPECTRA* [DISTANT] (HEMIPTERA: CICADELLIDAE) USING PARTIAL DNA SEQUENCE OF CYTOCHROME OXIDASE SUBUNIT I (COI) GENE

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ABSTRACT

The leafhopper Superfamily Membracoidea (Insecta: Hemiptera) is one of the most dominant groups of phytophagous insects. It comprises a total of 15,000 species worldwide and is very common in rice fields. *Cofana spectra* (Distant) is a pest of paddy, notably in upland rice fields, which suck sap from the leaves and results drying of leaf tips leading the leaf flip orange and curl. The species has been attained a major pest status in several districts of Tamil Nadu and Kerala of which very little work is available so far. Here we analyse the partial DNA sequence of cytochrome oxidase subunit I (COI) gene of *Cofana spectra* and its molecular phylogenetic status.

KEYWORDS: *Cofana spectra,* Cytochrome Oxidase Subunit I (COI) Gene, Molecular Phylogeny, Pest

INTRODUCTION

The leafhopper of Membracoidea (Hemiptera: Cicadellidae) are mainly the plant-feeding insects with more than 15,000 species described in the world (Dietrich et al., 2001). Some species are important pests of agriculture, forestry and industrial crops while some others are well-known as virus transmitters. White rice leafhoppers, *Cofana spectra* could be a tormentor of paddy that feeds on the sap matter leading to folding and drying of the leaf. This is a major tormentor of rice causing stunning yellowing of plants, and severe infestation terminating in plant death (Sam and Chelliah, 1984; Wilson and Claridge, 1991). Two species of the *Cofana* genus are common within the rice fields. Taxonomically *C. spectra* come under the family Cicadellidae, subfamily Cicadellinae and genus *Cofana*. The species is additionally recorded on variety of economic grass species at a distance from rice fields (Young, 1979; Dale, 1994). *C. spectra* is the largest among the leafhoppers occurring on rice.

They are easily distinguished by its larger size; the presence of a large, central, black spot on the vertex of the head toward the posterior margin; and the brown lines on the forewing. Adults of *C. spectra* are found on the lower surface of the leaves or at the base of the plant. Adults are attracted to light at night. Females oviposit by making a cut parallel to the long axis of the leaf with their saw-like ovipositors (**Figure 1**). The eggs are laid in rows of 10–15 across the slit at the base of the plant above the water level. The number of eggs laid per female is about 50 and they hatch in 5–12 days.

Figure 1: a) *Cofana spectra* Adult; b) Male Genitalia; c) Female Genitalia

Though the species is a vital agricultural pest, nymphs and female adults are very difficult for proper identification using conventional morphological analysis. Therefore, the DNA barcoding has to be done so as to resolve the problem. The mitochondrial cytochrome oxidase subunit I (CO I) are utilized in many molecular taxonomic studies for unambiguous species identification (Herbert et al., 2003). The present study reports partial sequence of its mitochondrial cytochrome oxidase subunit I gene and its phylogenetic status.

MATERIALS AND METHODS

Cofana spectra specimens were collected using the sweep technique with an insect net from many locations of Kerala. Collected adult specimens were identified morphologically. Sex organ dissections were made and examined once necessary to validate the identification of the specimen and they were transferred directly into 70% ethanol.

The genomic DNA of *C. spectra* was isolated using NucleoSpin® Tissue Kit (Macherey- Nagel, Germany). 2 ng of genomic DNA was amplified for COI gene using the forward primer, 5'- CACCTGATATAGCTTTCCCCCG-3' and reverse primer, 5'- AGCTCCTGCTAATACAGGTAAAG-3'. The PCR reaction mixture consisted of 2 ng of genomic DNA (1 µl), 1 µl each forward and reverse primers at a concentration of 10 µM, 2 µl of dNTPs (2 mM), 2 µl 10X reaction buffer, 0.20 µl *Taq* polymerase (5 U/µl) and 12.8 µl H_2O. The PCR profile consisted of an initial denaturation step of 5 min at 95°C, followed by 30 cycles of 10 sec at 95°C, 10 sec at 55°C and 1 min at 72°C and ending with a final phase of 72°C for 3 min. The PCR product was column purified using Gene JET™ PCR Purification Kit (Fermentas Life Science). The purified PCR product was sequenced at Sci Genom Laboratories Ltd., Cochin, Kerala. The sequences obtained were assembled by using ClustalW and the consensus was taken for the analysis. The nucleotide sequence was searched for its similarity. MEGA6 software was used for the phylogenetic tree construction and analysis (Tamura et al., 2011).

RESULTS AND DISCUSSIONS

The PCR of the COI gene fragment of *C. spectra* yielded a single product of 305 bp. The BLAST search using the sequence revealed that the sequence obtained in this study was novel (Gen Bank Acession No. KJ186109). The evolutionary divergence of *C. spectra* within Cicadellidae family is given in the **Table 1**.

Table 1: Evolutionary Divergence between COI Sequence of the Species *C. spectra* and Other Hemipterans

Sl. No.	Species name with GenBank Acession No.	% of Divergence
1	(HEQT022-08) *Cofana spectra*/Australia	1%
2	(HEQT051-08) *Cofana spectra*/Australia	1%
3	(AY959335) *Homalodisca liturata*/USA	17%
4	(HM891804) *Thricops diaphanus*/Canada	19%
5	(KC499838) *Pentacricia_aldrichii*	20%
6	(HM388867) *Hydrotaea anxia*	20%
7	(EU162460) *Onthophagus nuchicornis*	20%
8	(EF050557) *Brachypanorpa carolinensis*	20%
9	(KC499839) *Pentacricia aldrichii*	21%
10	(JF287190) *Eukiefferiella* sp.	21%
11	(HM388861) *Hydrotaea anxia*	20%
12	(FJ025614) *Haematobia irritans*	21%

The composition of nucleotides of *C. spectra* in each codon position was analysed and compared with other species of Hemiptera. The result indicates that the composition of each nucleotide in COI sequence of *C. spectra* showed similarity with other Hemipterans species. But the use of nucleotide in each codon position showed difference in the COI sequence of *C. spectra* compared to other Hemipteran species. Variation in the nucleotide sequence is a fundamental property of all living organisms which can be used for their identification and phylogenetic status. DNA barcoding provide rapid and automatable species identification by short standardized DNA fragment as species tag and its makes the Linnaean classification system more accessible (Herbert, 2005). The COI sequence obtained in this study showed significant variation with other species of the same family. The COI sequence can also be used for evolutionary studies and host insect relation studies of *C. spectra*.

NJ clustering analysis showed single monophyletic clade of the sequences belonging to the same species without any overlap, even though these sequences are from the specimens separated by a large geographic distances. The average nucleotide composition proportions for these twelve sequences were G, 14.6%; A, 29.7%; T, 41.1%; and C, 14.6%. The present results indicate that an identification system for insect life based on the COI gene will be highly effective. Although COI divergences appear too low to regularly enable species diagnosis within the insects, generic-level identifications in these organisms remain a prospect. More importantly, the mitochondrial genomes of closely allied species in other phyla, those that comprise the bulk of animal diversity, regularly show sufficient sequence diversity to enable their discrimination.

The evolutionary history of *C. spectra* was inferred using the Neighbour- joining method (**figure 2**). Phylogeneticaly *C. spectra* (HEQT022-08) from Australia is the nearest relative of *C. spectra* (Acession No. KJ186109) isolated from Kerala. The species *C. spectra,* which comes under the family Cicadellidae shows higher AT content. Gurney et al., (2000) reported that closely connected species shows 99% similarity within the standardized DNA sequence whereas distantly connected species will show 90% within the same sequence. The NJ clustering analysis showed inter and

intra species divergence. The intra species nucleotide divergence between *C. spectra* calculated shows 1% divergence with *C. spectra* from Australian region. Interspecies nucleotide divergence for 10 species shows divergence from 17-21%. This shows a deep interspecies nucleotide divergence. The partial sequence data of the COI gene for all 13 species of Transition/Transversion bias (R) is 0.51. There is no intraspecies peptide divergence between *C. spectra* from Kerala and Australia region. This peptide similarity shown in intraspecies between the fauna of India and Australia proved their common orgin from Gondwana land. After the separation, India continued its rapid northward migration and colliding with southern Asia (Barron and Harrison, 1980) and reached the tropical Asia by transportation from Gondwana on the Indian raft. The above peptide similarity indicate that the *C. spectra* may have as early as the origin of the supercontinent Gondwana which later spread to the adjacent countries after the Gondwana separation. Interspecies peptide divergence for 10 species shows divergence from 11-18%. Maximum transitional substitutions are C to T and transversionsal substitutions are A to T. It can be concluded that the COI sequence of *C. spectra* showed considerable variation with all other related species in the Hemipteran family, therefore the COI sequence identified in this study can use as barcode for identification of this insect at any stage of its life cycle.

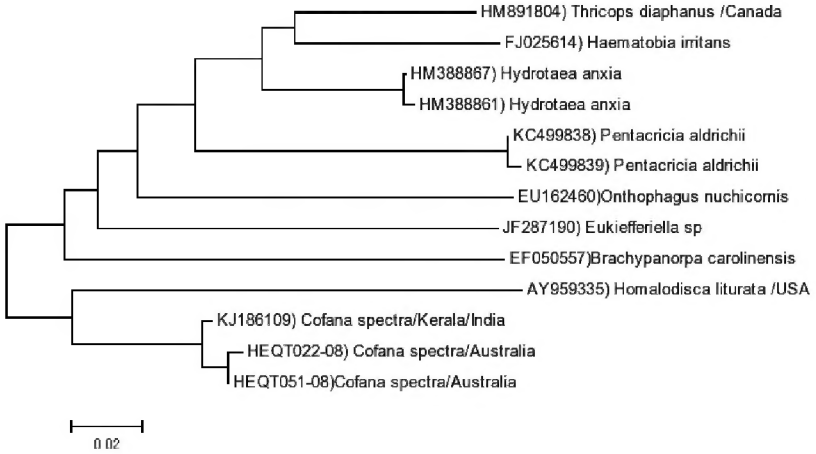

Figure 2: Phylogenetic Status of *C. spectra* in the Family Cicadellidae. The Evolutionary History was Inferred Using the Neighbour-Joining Method Using COI Partial Sequence

CONCLUSIONS

The present study on molecular evolutionary analysis using partial mitochondrial cytochrome oxidase subunit I (COI) gene sequence explicates phylogenetic relationships of *Cofana spectra*. The study suggest that the best phylogenetic inferences can be created through moderately divergent nucleotide data from mitogenomes, of which the COI gene is best suited for deciphering the Hemipteran taxonomic levels.

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